Functional, Hemodynamic, and Anatomic Changes in Isolated Perfused Dog Lungs: The Importance of Perfusate Characteristics

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STUDIES of isolated perfused mammalian lungs have been performed for many reasons,[4, 5, 7, 9, 17, 18, 28, 29] yet early deterioration of the perfused lungs remains poorly understood. Among factors known to be influential are three characteristics of the perfusate: age of the blood, anticoagulants, and perfusion temperature. These factors, while probably not the only ones involved in pathologic changes, are of such importance that under specifically defined perfusate conditions, it is possible to perfuse lungs up to 6 hours with preservation of pulmonary function, hemodynamics, and morphology.[26]

The present study was designed to evaluate systematically relative contributions of each of these three factors to the process of deterioration of perfused lungs. Results have relevance not only to lesions which develop in isolated perfused lungs, but also to those that occur in humans subjected to cardiopulmonary bypass. Furthermore, the sequence of events during deterioration of isolated perfused lungs and perfusate conditions associated with damage provide clues to pathogenesis.

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Methods

Sixty-four lung perfusions were performed using the method diagrammed in Figure 1 and described previously.[26] Briefly, the pulmonary artery and left atrium were cannulated with the lungs *in situ*. The perfusion circuit included a heat exchanger and a bubble-type gas exchanger through which 90% nitrogen and 10% carbon dioxide were passed to remove oxygen and add carbon dioxide to the perfusate. The lungs were ventilated with ambient air at a rate of 14 inflations per minute and a stroke volume of 400 cc. The extracorporeal system (in which all tubing used was of disposable sterile plastic), was filled with 1,500 ml. of blood. Pulmonary blood flow, oxygen and carbon dioxide tensions, pH, and blood pressures in the pulmonary artery and left atrium were measured in the intact animal prior to and at regular intervals during perfusion. Intratracheal pressure was similarly monitored. From these measurements pulmonary vascular resistance, compliance,** and pulmonary ar-

** Compliance in these experiments was calculated from the stroke volume of the respirator and the net tracheal pressure change with each inspiration. It is expressed as liters/cm. of H_2O and is inversely related to tracheobronchial resistance.

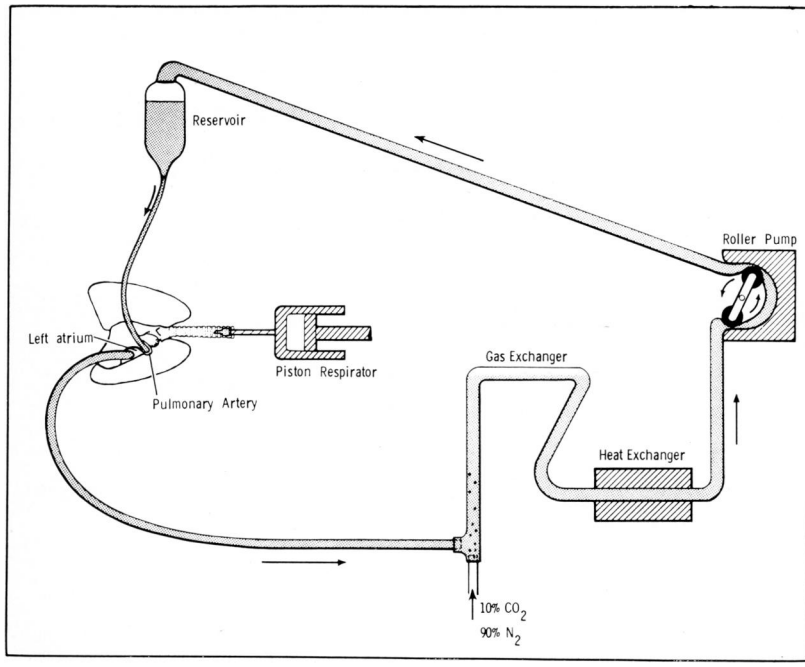

FIG. 1. The system used for isolated lung perfusion. Lungs remained within the opened thorax during perfusion.

teriovenous oxygen and carbon dioxide differences were calculated.

Lungs were observed during perfusion after which they were inspected, photographed, fixed, and sectioned as previously described.[26]

Ten perfusions were conducted with sterile technic. At the conclusion of these, perfusate cultures were incubated for 72 hours in thioglycollate broth and 1 ml. aliquots were spread on blood agar and tryptocase soy agar plates for colony counts at 24 and 48 hours. The remaining perfusions were conducted with clean, but not sterile, technic.

Blood for perfusion was obtained by a femoral artery cannula from healthy mongrel dogs anesthetized with intravenous thiopental sodium. Blood was collected under sterile conditions in plastic bags containing either 75 ml. of formula A acid-citrate-dextrose (ACD) solution, or 4,000 U.S.P. units of heparin sodium. Clotting of stored heparinized blood was prevented by the addition of 5,000 units of heparin sodium on the third, sixth, and ninth day of storage. All stored blood was refrigerated at 4° C.

Perfusate conditions in the experimental groups are summarized in Table 1. In 32 experiments, homologous perfusate was anticoagulated with ACD solution (Table 1, Groups 1–4). The lungs in Group 1 experiments were perfused at 25° C. with blood less than an hour old; in Group 2, the lungs were perfused at 25° C. with blood 21 days old. In Group 3 they were perfused at 38° C. with blood drawn less than an hour before, and in Group 4, lungs were perfused at 38° C. with blood 21 days old.

In 24 other perfusion experiments with homologous blood, heparin was used as anticoagulant (Table 1, Groups 5–8). The lungs in Group 5 were perfused at 25° C. with blood drawn less than an hour before. In Group 6, lungs were perfused at 25° C. with blood 21 days old. Lungs in Group 7

In the pure sense, compliance refers to the volume/pressure relationships at all degrees of inflation without flow. Since gas flow was zero only at the end of inspiration and expiration in our experiments, our calculations were a limited reflection of compliance in the pure sense.

TABLE 1. *Details of Perfusions in Experimental Groups*

Group Number	Perfusate Source	Anticoagulant	Perfusate Temperature (°C.)	Perfusate Age (in days)	Number of Experiments
1	Homologous	ACD	25	<1	8
2	Homologous	ACD	25	21	8
3	Homologous	ACD	38	<1	8
4	Homologous	ACD	38	21	8
5	Homologous	Heparin	25	<1	6
6	Homologous	Heparin	25	21	6
7	Homologous	Heparin	38	<1	6
8	Homologous	Heparin	38	21	6
9	Autologous	ACD	25	5–20*	2
10	Autologous	ACD	38	5–20*	2
11	Autologous	Heparin	25	5–20*	2
12	Autologous	Heparin	38	5–20*	2

* Autologous blood drawn in 350 ml. aliquots 5, 10, 15 and 20 days prior to perfusion.

were perfused at 38° C. with blood drawn less than an hour before, and in Group 8, lungs were perfused at 38° C. with blood 21 days old.

In four perfusions with heparinized homologous blood, samples of perfusate were drawn at the beginning and end of perfusion for determinations of hematocrit, leukocyte count, blood glucose, serum hemoglobin, and serum sodium, potassium, and chloride. In six perfusions conducted with ACD anticoagulated blood, similar tests were performed.

Eight additional perfusions were conducted with autologous blood perfusate under conditions shown in Table 1 (Groups 9–12). For each of these, 1,500 ml. of blood were obtained under sterile conditions in 350 ml. aliquots, 20, 15, 10, and 5 days prior to perfusion and refrigerated at 4° C. until use.

One hundred-twenty-minute perfusions were planned in all groups. Deterioration of the lungs, as indicated by progressive loss of perfusate into the lungs and the appearance of bloody froth in the respirator, forced early termination of several experiments in some groups. Times of forced termination were noted and provided an objective, statistically analyzable criterion of perfusion damage. It should be noted that an upper limit of 120 minutes was imposed in these experiments. A finer discrimination among groups by this criterion could have been obtained by carrying all perfusions to a point of forced termination.

Lung damage was graded according to the following criteria. Grade 0 lungs were grossly and microscopically normal; after

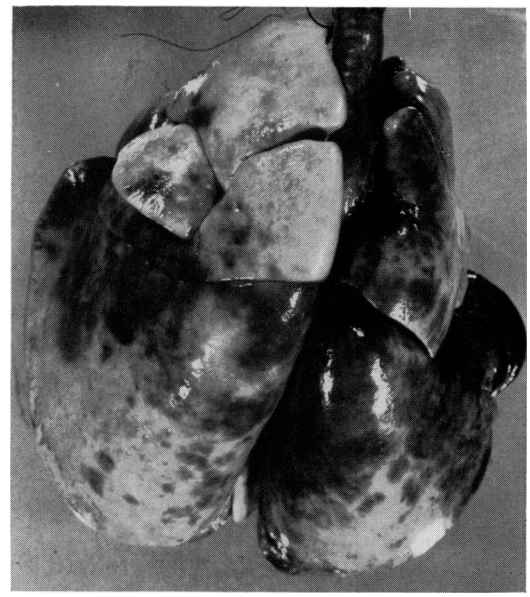

FIG. 2. Severe hemorrhagic lesion on surface of partially inflated intact lungs which had typical Grade 4 damage.

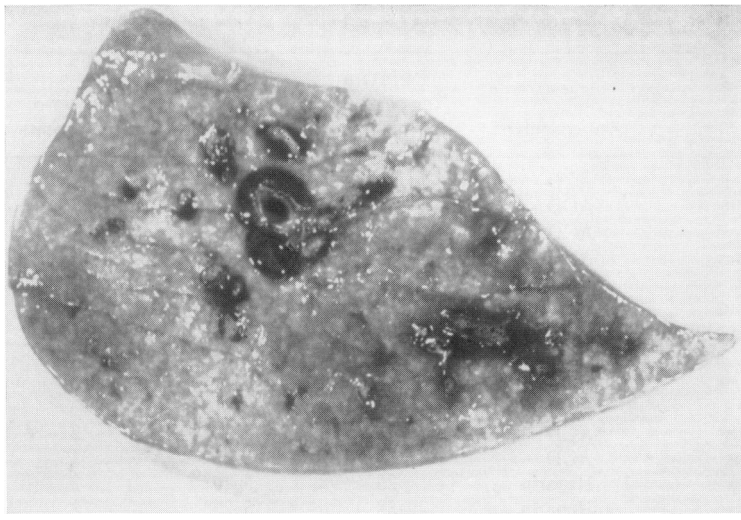

FIG. 3. Cut surface of a lung with Grade 3 damage. Note peri-arterial and peribronchial hemorrhage (×3).

120 minutes of perfusion, there was no subpleural or peri-arterial hemorrhage, pulmonary edema, or intrabronchial froth. Grade 1 lungs had one to four subpleural hemorrhages, all less than 1 cm. in diameter, but were otherwise as in Grade 0. Grade 2 lungs had focal subpleural or small peri-arterial hemorrhages or slight pulmonary edema, but were without confluent areas of parenchymal hemorrhage or

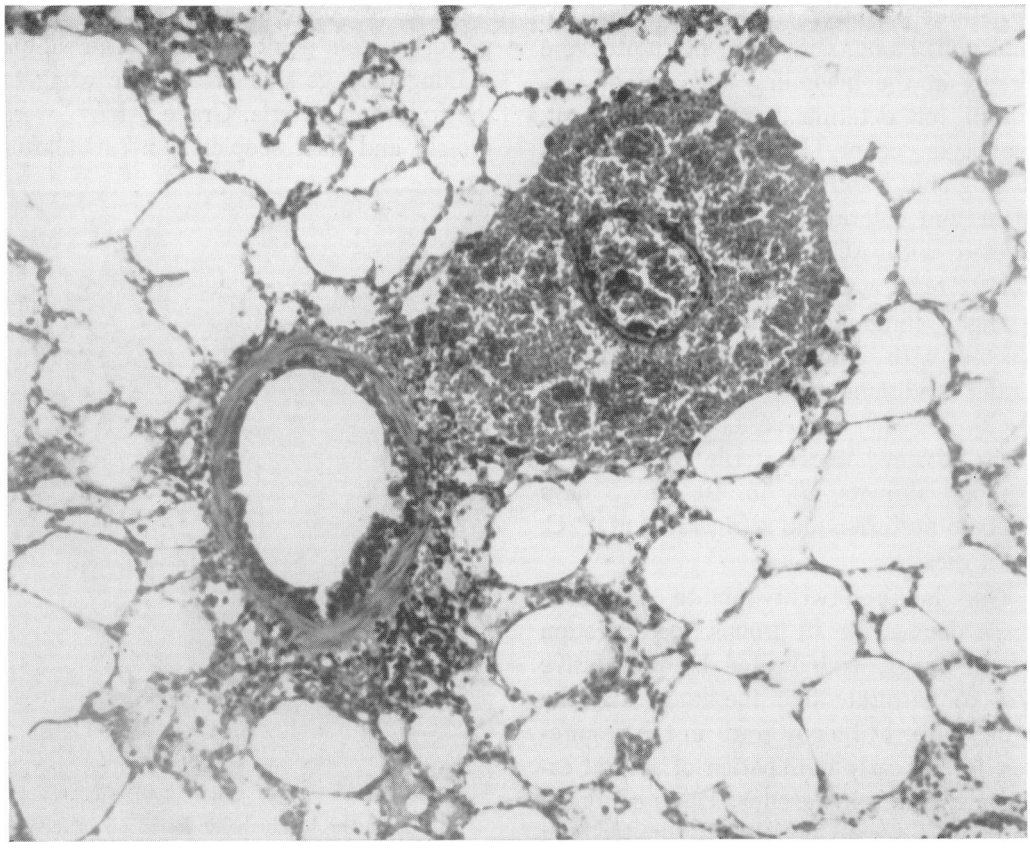

FIG. 4. Lung showing hemorrhage around small muscular artery (×140, hematoxylin and eosin).

edema. Group 3 lungs had peri-arterial hemorrhage involving one-fourth to one-half of the arteries, moderate pulmonary edema, areas of confluent parenchymal hemorrhage greater than 3 cm. in diameter, or some intrabronchial froth in any or all combinations. Grade 4 lungs had perivascular hemorrhage involving more than two-thirds of the vessels, subpleural areas of confluent hemorrhage covering one-fourth to one-third of the surface of the lungs or intratracheal hemorrhagic froth. All lungs were graded without knowledge of termination times. This system provided an alternative approach for statistical analysis of the data. It was found that early termination time bore a parallel relationship to the grade of pulmonary damage. For example, all perfusions resulting in lung damage classified as Grade 4 had been terminated early.

Results

1. Gross and Microscopic Changes in Perfused Lungs. When changes in lungs from all groups were considered together, a spectrum of findings was present. At one end, lungs were normal grossly and microscopically and free of hemorrhage, edema, and atelectasis. At the other end of the spectrum, there were changes characterized most prominently by varying degrees of focal hemorrhage. Involvement on the pleural surface was patchy and varied, ranging from a few square millimeters to almost an entire lobe. On the surface of the intact lung, lesions were depressed, dark, atelectatic areas surrounded by normal lung tissue (Fig. 2). These areas collapsed readily when the inflating pressure was released and could only be reinflated with difficulty. Pleural hemorrhage over 1

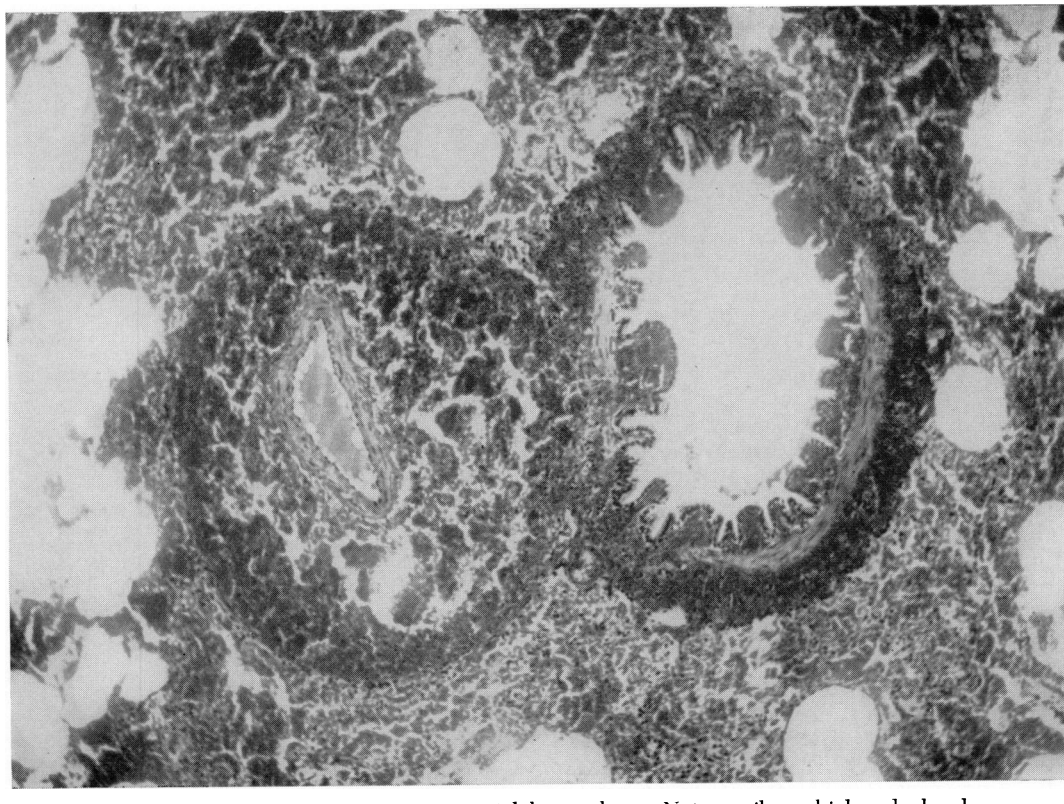

FIG. 5. Lung showing severe peri-arterial hemorrhage. Note peribronchial and alveolar extension of the hemorrhage (×90, hematoxylin and eosin).

cm. in diameter invariably reflected extensive intrapulmonary hemorrhage which, in early forms, obviously surrounded pulmonary arteries and bronchi (Fig. 3). When severe most of the parenchyma of a lobe was affected. There was no predilection for any particular lobe and severely altered and relatively uninvolved lobes commonly coexisted. When parenchymal hemorrhage was severe, frothy bloody fluid filled the bronchi and trachea.

Often, but not invariably, pulmonary edema accompanied these hemorrhages. Grossly, edema was usually confined to the posterior or dependent portions of the lower lobes which were heavy and hypocrepitant. Edema usually involved all or most of a lobe and tended to be bilaterally symmetrical. When intratracheal pressure was released, edematous lobes did not collapse. Blood-tinged fluid oozed from cut surfaces, and parenchymal hemorrhage was a prominent associated feature.

Microscopically, peri-arterial hemorrhages were found even in minimally involved portions of affected lungs in all groups. These hemorrhages existed around arteries of all sizes, including those less than 50 μ in diameter and the right and left main pulmonary arteries. Hemorrhages appeared to originate from small muscular arteries around which extravasation of erythrocytes was noted even in minimally damaged lungs (Fig. 4). As the severity of the damage increased, the hemorrhage extended to involve adjacent bronchi and alveoli (Fig. 5). The changes were definitely focal. In some areas, alveoli were completely filled with erythrocytes. Subpleural alveoli were usually spared, except in the most severe

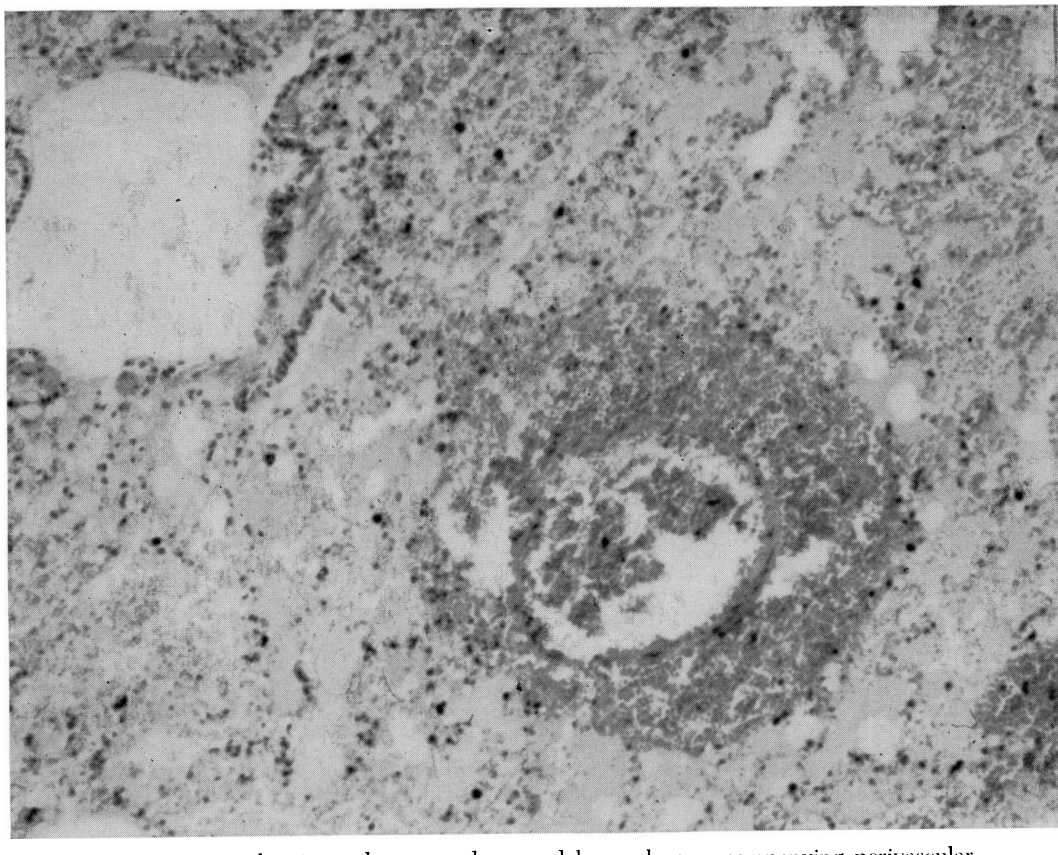

FIG. 6. Lung showing pulmonary edema and hemorrhage accompanying perivascular hemorrhage (×140, hematoxylin and eosin).

TABLE 2. *Relationship of Pulmonary Damage to Perfusion Characteristics with Homologous Perfusate*

Group Number	Perfusate Conditions	Number of Experiments	Average Grade of Lung Damage*	Number in which Early Termination Forced	Termination Time in Minutes Average-Range
1	ACD, 25° C., <1 hr.	8	0 (0)	0	120
2	ACD, 25° C., 21 d.	8	3 (1–4)	2	104 (56–120)
3	ACD, 38° C., <1 hr.	8	3 (2–4)	3	100 (75–120)
4	ACD, 38° C., 21 d.	8	4 (3–4)	4	92 (45–120)
5	Heparin, 25° C., <1 hr.	6	2 (1–4)	1	111 (64–120)
6	Heparin, 25° C., 21 d.	6	1 (0–3)	0	120
7	Heparin, 38° C., <1 hr.	6	2 (1–3)	0	120
8	Heparin, 38° C., 21 d.	6	4 (3–4)	4	109 (80–120)

* Criteria for grading lung damage described in text. Range of grades of lung damage within groups shown in parenthesis.

lesions. Alveoli subjacent to the affected areas often were normal or contained light- or dark-staining eosinophilic homogenous material (edema) with a variable number of erythrocytes (Fig. 6). Some alveoli adjacent to areas of hemorrhage were compressed, while others were hyperinflated, and capillaries in all areas of the lung were normal. Microemboli or microthrombi were not identified, and there was no evidence of engorged bronchial vessels or lymphatics. Rarely was margination of neutrophils present in arteries.

2. Relationship of Pulmonary Changes to Perfusate Characteristics is summarized for homologous perfusate in Table 2. With ACD anticoagulant, perfusion at 25° C. with blood less than an hour old produced no damage (Group 1). When ACD perfusate was 21 days old or was perfused at 38° C., significant pulmonary damage resulted (Groups 2 and 3). Perfusate age and a higher perfusion temperature were additive with regard to the severity of lung damage produced (Group 4). Although there was some variation within groups, these differences, when analyzed by the chi-square test on the basis of grades, were significant ($p < 0.001$). This same result was obtained in a variance analysis utilizing the time to termination of perfusion as the criterion.

With heparin as anticoagulant (Table 2, Groups 5–8), all conditions of perfusion resulted in pulmonary damage in some perfusions. Somewhat paradoxically, lungs perfused at 25° C. with 21-day-old blood (Group 6) appeared to be damaged least. This finding was of borderline significance ($0.1 > p > 0.05$) when tested by grade of pulmonary damage and did not reach a level of significance when examined by the criterion of forced termination times ($p > 0.2$). When 21-day-old blood was used in perfusions conducted at 38° C. (Group 8), the greatest degree of lung damage resulted. This finding could not be established as significant by either criteria. The finding of some lung damage in all of these groups tends to obscure the real differences, if any, among them. It should also be noted that in Groups 5, 6, and 7 occasional two-hour perfusions were performed with almost no detectable anatomic pulmonary changes.

The relationship between perfusion conditions and the severity of pulmonary damage when autologous blood was used as the perfusate is summarized in Table 3. When heparin was used as anticoagulant or perfusions were performed at 38° C., pulmonary damage resulted in all instances. Only when ACD mixture was the anticoagulant agent and when the lungs

TABLE 3. *Relationship of Pulmonary Damage to Perfusion Characteristics with Autologous Perfusate. Each Group Comprised of Two Experiments*

Group Number	Perfusate Conditions*	Average Grade of Lung Damage**	Number in Which Early Termination Forced
9	ACD, 25° C.	0	0
10	ACD, 38° C.	3	1
11	Heparin, 25° C.	3	1
12	Heparin, 38° C.	3	0

* Autologous blood for perfusate was drawn in 350 ml. aliquots, 20, 15, 10, and 5 days prior to perfusion.
** Criteria for grading lungs described in text.

were perfused at 25° C., was there no pulmonary damage.

3. Sequence of Hemodynamic and Functional Changes in Perfused Lungs. Pulmonary vascular resistance, flow, perfusate levels, compliance, and arteriovenous oxygen and carbon dioxide differences were remarkably stable when lungs showed no morphological damage.[26] In contrast, appreciable functional and hemodynamic alterations occurred in perfusions in which gross and microscopic anatomic lung damage was evident. A typical sequence of changes is shown in Figure 7. Increased pulmonary vascular resistance with a rise in pulmonary artery pressure

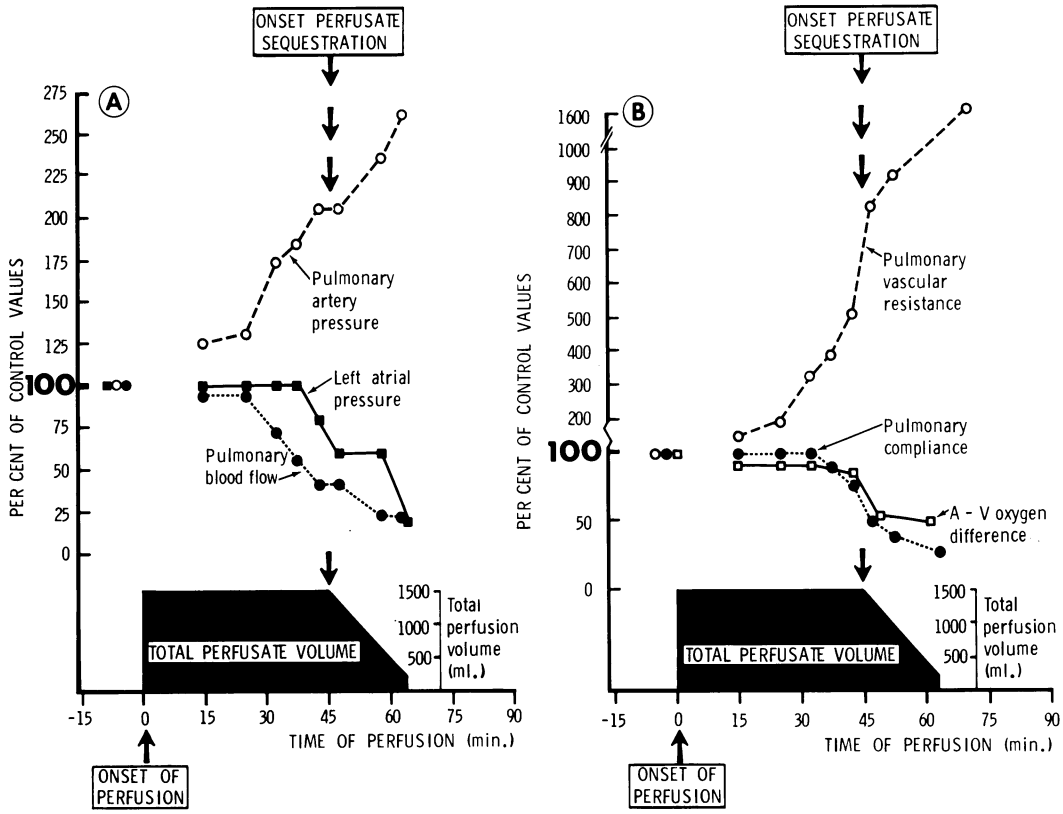

FIG. 7. Sequence of functional and hemodynamic changes in typical perfusion resulting in Grade 4 morphologic damage. Pressures and flow shown in A. Pulmonary vascular resistance, compliance and arteriovenous oxygen difference shown in B.

and a decrease in blood flow were the earliest changes noted. As pulmonary vascular resistance increased, it was accompanied by a fall in left atrial pressure. Within 15 to 45 minutes of these changes, decreased compliance and arteriovenous oxygen and carbon dioxide differentials were noted. Ten to twenty minutes later, there was loss or sequestration of perfusate in the lungs. Following this, in most perfusions with severe anatomic damage, progressive deterioration of all parameters continued until loss of perfusate or intratracheal hemorrhagic froth forced termination of the perfusion.

4. Correlation of Perfusate Cultures with Lung Damage. Of the ten perfusions conducted under sterile conditions and monitored by terminal perfusate cultures, five resulted in severe lung damage and five produced none. There was no correlation between pulmonary damage and bacterial growth. Perfusate from four perfusions associated with the most severe pulmonary damage was sterile, whereas the perfusate from three perfusions in which there was no pulmonary damage had up to 30 colonies per ml. of multiple organisms including *E. coli, Proteus sp., S. aureus* and *S. albus.*

5. Correlation of Hematological and Chemical Changes in Blood with Pulmonary Damage. Hematocrits, leukocyte counts, blood glucoses, and serum hemoglobins and electrolytes were measured in four experiments conducted with heparinized perfusate and in six conducted with ACD perfusate. Three of the four heparin perfusions and four of the six ACD perfusions resulted in severe pulmonary damage. In these ten perfusions, there was no correlation between the pulmonary damage and changes in these parameters.

Discussion

These results show that perfusate conditions can influence the integrity of isolated lungs perfused by a system which includes a bubble-type gas exchanger. Since isolated perfused lungs and lungs from patients dying after cardiopulmonary bypass from so-called post-perfusion pulmonary damage have similar pathologic changes,[2, 16, 20, 25, 27] the two conditions may have a common etiology. Therefore, it would appear desirable in cardiopulmonary bypass procedures to maintain the same conditions as those which minimize damage to isolated perfused lungs. This is particularly important in procedures requiring protracted use of a pump-oxygenator. The most important of these conditions in the isolated lung perfusions has been a low perfusion temperature. Indeed, recent lower incidence of postperfusion pulmonary damage may be related to the more widespread use of hypothermia in protracted open-heart operations as well as to other factors such as hemodilution.[12, 20, 30] On the basis of the data from these studies of isolated perfused lungs, only fresh, ACD blood was consistently associated with minimal lung damage. Although conclusions based on extrapolation of these results to human cardiopulmonary bypass are not justified from our data, it is interesting that others have advocated the use of ACD blood in open-heart operations for other reasons.[13, 23]

Since ischemic deterioration is generally delayed by hypothermia, the protective effect of a lower perfusion temperature could be explained if the lesion were a manifestation of ischemia to those portions of the lung served by bronchial circulation which is obviously arrested during perfusion. However, it is unlikely that the lesion occurs on this basis since similar pathologic changes are produced by other circumstances in which no interference with bronchial circulation occurs.[2, 20, 21, 27] Alternatively, correlation between perfusate temperature and lung damage suggests that some chemical process, possibly enzymatic, accounts, in part at least, for the deterioration. The effect of temperature and the occurrence of the damage in our

perfusion system, without prior artificial changes in the inflow pressure head or the resistance to outflow, suggest that production or destruction of some mediator or factor plays an etiologic role in producing pulmonary vascular changes which result in the lung lesions seen. The mechanism of release or destruction of such a factor remains unknown. However, its complexity is emphasized by the inconsistent and paradoxical observations in groups perfused with heparinized blood. It is attractive to speculate that liberation of a proteolytic substance is responsible for lysis of the walls of pulmonary arteries or their vasa vasorum, thereby producing the peri-arterial hemorrhages.

Differences in lung damage that resulted when the two anticoagulant agents were used for the perfusate are of questionable significance except in the fresh 25° C. perfusions (Group 1 v. Group 5). These differences, if confirmed, would be difficult to explain and also emphasize the complexity of the pathogenesis of the lung damage. The differences may be due to a protective action of glucose on cells or proteins under certain conditions. Alternatively, heparin, which has extensive anti-enzymatic effects,[3] may influence chemical reactions that result in the production of a lung-damaging factor or in the destruction of a lung-protective moiety.

An identical sequence of pathophysiologic events and pathologic lesions resulted when autologous and homologous perfusates were used under comparable conditions. This is of special interest because homologous blood has been implicated frequently as a cause of post-perfusion pulmonary damage in patients.[10, 16, 21]

Some effect of a gas exchanger, such as cell lysis or protein denaturation,[2, 14, 16] may be a part of the lung-damaging mechanism in the isolated perfusion system. However, it is possible that a related mechanism results in other forms of histologically similar pulmonary damage such as that seen after hypovolemic shock with or without retransfusion.[8, 15, 19, 22] Furthermore, the gas exchanger cannot be the sole factor causing pulmonary damage, since perfusions conducted at 25° C., with fresh ACD blood failed to produce lung injury although the circuit included a gas exchanger; and similar pulmonary changes have been noted in lungs perfused by circuits not containing a gas exchanger.[1, 25]

The sequence of hemodynamic and functional events in deteriorating perfused lungs may provide a clue to the mechanism of injury. The earliest change is a rise in pulmonary arterial pressure, despite a constant inflow pressure head. This suggests that vasoconstriction occurs proximal to the capillary bed since venoconstriction would not only raise the pressure in the pulmonary artery but would cause a simultaneous loss of perfusate volume into the lungs. This arterial constriction may be functional, reversible, and the cause of ischemic vascular damage and hemorrhage. More probably, this constriction is due to peri-arterial hemorrhage, the earliest anatomic change seen. Only serial biopsies correlated with pressure changes will determine whether the hemorrhage is a cause or an effect of the arterial constriction.

When peri-arterial hemorrhage spreads around respiratory bronchioles and into alveoli, gas exchange is decreased and the lungs become less compliant because of alveolar surface tension altering qualities of blood[24] and mechanical obstruction and compression of the smaller air passages and alveoli. As the hemorrhage continues, perfusate is lost into the lung parenchyma, alveolar lumens, and ultimately, into the bronchi and trachea.

Other investigators implicated various mechanisms in the pathogenesis of damage to perfused lungs.[1, 4, 5, 25, 28, 29] Trowell studied the histological changes in isolated perfused lungs and noted pulmonary edema with alveolar exudate and lymph-

atic distension, peri-arterial hemorrhage, and collections of polymorphonuclear leukocytes in the small pulmonary blood vessels. He postulated that the peri-arterial hemorrhage resulted from rupture of the vasa vasorum secondary to backflow into and distension of the bronchial circulation. He proposed that distension and rupture of peri-arterial lymphatics was an alternate mechanism.[25] Yong and co-workers speculated that high serotonin or histamine levels resulted in increased pulmonary vascular resistance and deterioration of perfused lungs, but they were unable to document any relationship between pulmonary vascular resistance and levels of these vasoactive substances.[29] Awad and associates speculate that changes in alveolar surface active material (surfactant) underlie the damage occurring in isolated perfused lungs,[1] and Gardner demonstrated changes in the surface tension of pulmonary extracts following extracorporeal pump-oxygenator bypass.[11] However, doubt is cast on the primary or causal nature of these changes by the early occurrence of peri-arterial hemorrhage without alveolar changes in our perfused lungs. Whatever the exact pathogenesis of the pulmonary damage, its elucidation is warranted by its potential significance in postperfusion and possibly other forms of lung damage.

Summary

The importance of perfusate temperature (38° C. v. 25° C.), age (1 hour v. 21 days), anticoagulant (ACD v. heparin), and source (homologous v. autologous) has been evaluated in 64 isolated lung perfusion experiments.

Morphologic, functional, and hemodynamic changes did not occur with fresh, ACD, 25° C. perfusions, but did occur in varying degrees under all other circumstances of perfusion. In general, 25° C. perfusions resulted in less damage than comparable ones at 38° C. With ACD perfusate, age and temperature were additive in producing lung damage. With heparinized perfusate, differences in results were not striking although 25° C. perfusions with 21-day-old blood produced the least pulmonary damage and 38° C. perfusions with 21-day-old blood appeared to produce the most. Autologous perfusate produced lung damage equivalent to homologous perfusate under comparable conditions. No correlation could be made between the pulmonary damage and changes in perfusate bacterial growth, hematocrit, leukocyte count, blood glucose, serum hemoglobin, or serum electrolytes.

The pulmonary lesion was characterized pathologically by peri-arterial and parenchymal hemorrhage with associated alveolar collapse and, in some instances, edema. These changes were associated first with elevated pulmonary artery pressure and decreased flow, second with decreased compliance and decreased gas exchange, and third with loss of perfusate volume into the lungs. This sequence suggests that precapillary vasoconstriction is the first and perhaps the basic alteration in the lungs.

Although the exact mechanism underlying damage in isolated perfused lungs remains obscure, the perfusate conditions causing changes suggest that it is complex and may be mediated by chemical or enzymatic reactions. Since this mechanism may be active in producing postperfusion pulmonary damage, it is logical that protracted pump-oxygenator procedures be conducted, if possible, under hypothermia using fresh ACD blood when needed for prime and replacement. Because a similar mechanism may cause pulmonary damage in other clinical situations, its further exploration and exact definition is warranted.

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